Int. J. Pharm. Investigation, 2021;11(2):230-237 *Original Article*

Formulation, Optimization and *in vitro* Evaluation of Apremilast Nanoemulgel for Topical Delivery

Sindhoor Shridharan Mulleria, Koland Marina*, Sawan Mukund Ghetia
NITTE (Deemed to be University), NGSM Institute of Pharmaceutical Sciences (NGSMIPS), Department of Pharmaceutics, Deralakatte, Mangalore, Karnataka, INDIA.

ABSTRACT

Background: Psoriasis is an autoimmune-mediated disease characterized marked by thickened plaques and erythematous skin, which can be treated by topical delivery of apremilast. The objective of this study was to prepare hydrogel-based nanoemulsions of apremilast for psoriasis therapy with a view of improving its solubility and permeability. **Methods:** Nanoemulsion components were chosen based on their solubility. Spontaneous emulsification technique was used to formulate apremilast loaded nanoemulsions D-optimal design was used to optimize the formulations. The optimized formulation was assessed for globule size, Polydispersity Index, percentage transmittance, entrapment efficiency, pH, and Transmission Electron Microscopy The optimized nanoemulsion was converted into gels using carbopol 940 and evaluated for critical parameters like spreadability, viscosity and extrudability. *In vitro* drug release and *ex vivo* permeation studies were conducted to understand the release kinetics and extent of permeation. **Results:** Particle size of the nanoemulsions were found to be in the range of 141 nm- 245nm. Transmission electron microscopic images of the optimised formulation showed spherical particles in the nano range. Optimised formulation exhibited excellent entrapment efficiency of 86% and was found to be thermodynamically stable. *In vitro* drug release studies of the nanoemulgel using the Franz diffusion cell apparatus appeared to follow zero-order kinetics. *Ex vivo* permeation through porcine skin showed substantial improvement in flux, permeability coefficient, and drug deposition in the skin in the case of nanoemulgel compared to drug dispersion. **Conclusion:** The results obtained from the studies demonstrated the immense potential of the developed formulations for topical application in psoriasis.

Key words: Psoriasis, Flux, Permeability, Deposition, Optimal, Surfactant.

Correspondence

Dr. Marina Koland
Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore-575018, Karnataka, INDIA.
Email: marinakol@nitte.edu.in

DOI: 10.5530/ijpi.2021.2.41

INTRODUCTION

Psoriasis is an autoimmune-mediated disease marked by excessive proliferation and incomplete differentiation of keratinocytes and is multifactorial in origin. It is characterized by the occurrence of thickened plaques and erythematous skin.[1,2] Treatment strategies include topical therapy like Vitamin D, topical calcineurin inhibitors, retinoids and coal tar to manage mild to moderate psoriasis and systemic treatments using methotrexate, cyclosporine, acitretin, and phototherapy for severe psoriasis. However, none of these treatments can completely cure the disease and is associated with severe side effects resulting from prolonged treatment. Biologic therapies, on the other hand, though useful, have their disadvantages related to treatment resistance, hospital admission, parenteral administration, adverse effect profile, expenses and management requiring a specialist setting.[3,4] Therefore, there is ongoing research for the discovery of an ideal drug for managing psoriasis.

Apremilast (APM) is a phosphodiesterase 4 inhibitor approved for the treatment of psoriasis or psoriatic arthritis worldwide. It is expected that apremilast could revolutionize the treatment of psoriasis since it does not interfere with immune suppression. Instead, it targets the central inflammatory signalling pathways and modulates the expression of various inflammatory mediators involved in this process.[5] Despite its good therapeutic effect, oral administration of apremilast causes several side effects such as diarrhoea, nausea, nasopharyngitis and abdominal pain, which lead to failure to adhere to the medication.[6] The topical application directly to the affected skin will avoid the problems associated with oral administration, and also dose reduction is possible since the first-pass metabolism can be bypassed.[7] However, apremilast's unfavourable physicochemical properties like low water solubility and low permeability hinder its topical application. Traditional dosage forms like cream and ointment are suitable only for short-term therapies and cannot permeate deeper skin layers. Nanoemulsions are transparent, kinetically stable systems composed of two immiscible phases with droplet size between 20–500 nm.[8] These submicron emulsions permeate the rugged plaques of psoriatic skin by swelling and extracting the skin's lipids, thereby improving permeation through the pores.[9] Hence, considering the physicochemical limitations of apremilast, challenges associated with psoriatic skin, oral delivery, an attempt is made to develop an effective topical formulation of apremilast in the form of a nanoemulsion based gel.

MATERIALS AND METHODS

Apremilast was received as a gift sample from Glenmark Life Sciences Ltd, Ankleshwar, Gujarat. Sefsol 218, Sefsol 228, Transcutol P were collected from Gattefosse, France. Tween 80, propylene glycol, tween 20, oleic acid, olive oil, PEG 400, Isopropyl alcohol, n butanol were procured from Himedia Laboratories Pvt. Ltd. Isopropyl myristate (IPM) was purchased from Loba Chemie Pvt. Ltd., India. Ultra-pure deionized water was used throughout the experiments. All other solvents and chemicals used were of analytical grades or higher.

METHODS

Solubility studies of apremilast in oils and surfactants

The solubility of apremilast in various oils, surfactants, and co-surfactants was measured by adding an excess quantity of drug in 2 ml of the chosen vehicle and transferred to a shaking water bath maintained at 25±2°C for 72h. The samples were centrifuged at 10,000 rpm for 10 min to eliminate the undissolved apremilast and filtered using Whatman filter paper for quantification using UV-spectrophotometer at 230 nm.[10,11]

Development of pseudo ternary diagrams

The phase diagrams were constructed using oil, surfactant, and co-surfactant selected from the solubility tests. Initially surfactants and co-surfactants were combined in various weight ratios to form S_{mix} (1:0,1:1, 2:1,3:1, 1:2 and 1:3 % w/w). The oil and the specific S_{mix} (ratio of surfactant and co-surfactant) were then added in weight ratios ranging from 1:9 to 9:1 % w/w. The resultant mixtures were subsequently titrated using distilled water as the aqueous phase with mild stirring until equilibrium. The nanoemulsions were vortexed and observed for any phase separation for 24hr.[12]

Thermodynamic stability studies

The nanoemulsions were subjected to six alternating heating (45°C) and cooling cycles (4°C) with a period of 48h for each process. Nanoemulsions that passed the test above were centrifuged for 30 min at 3500 rpm. The formulations that overcome all these tests were regarded as thermodynamically stable.[13]

Preparations of nanoemulsions

Apremilast nanoemulsions were prepared using spontaneous emulsification method. The drug was solubilized in sefsol 218, then S_{mix} and water were added, appropriately mixed, and stirred at room temperature on a magnetic stirrer.

Optimization of apremilast nanoemulsion by D-optimal statistical design

Considering the solubility and the amount of drug needed to be added to the nanoemulsion, various combinations of oil, water, and S_{mix} were selected from the ternary phase diagram within the nanoemulsion area and further optimized using D-optimal statistical design. The 16 trials generated using D optimal Design is given in Table 1.

Characterization of optimized formulation

Particle size and size distribution

The particle size and PDI of the final nanoemulsion were analyzed using Malvern zeta sizer. All measurements were performed at 25 ± 1°C with a scattering angle of 90°C.

Zeta potential determination

Zeta potential of the nanoemulsion was measured using Zetasizer (Malvern instrument, Westborough, MA) using disposable Zeta cell at 25°C.

Transmission electron microscopy

The shape and morphology of optimized nanoemulsion were examined using TEM. The sample was stained with 1% w/v aqueous phosphor-tungstic acid solution and then observed under the microscope.[14]

Percentage transmittance

Nanoemulsion clarity was evaluated by measuring the percentage transmittance through UV Visible spectrophotometer (UV-1800 Shimadzu, Japan) at 650 nm.

Table 1: D-optimal design generated for using Design-Expert software nanoemulsion formulations.

S.No	Independent variables			Responses		
	A:Smix(%)	B:Water(%)	C:Oil(%)	Particle size(nm)	Transmittance(%)	Solubility(mg/ml)
1	52.1185	27.6437	20.2378	172.3	96.11	269.5
2	55	20	25	205.6	97.89	296.5
3	33.7366	41.2634	25	239.6	92.11	273.5
4	25	60	15	245.1	88.91	248.8
5	60	25	15	145.1	99.01	271
6	60	25	15	141.7	99.51	268.2
7	49.8438	35.1562	15	179.7	95.24	257.6
8	34.3794	50.6206	15	226.2	90.81	250.9
9	43.2222	31.7778	25	231.8	93.31	287.5
10	25	50	25	258.2	91.16	264.7
11	60	20	20	156.8	98.15	286.5
12	25	50	25	253.2	91.96	261
13	25	60	15	241.7	88.05	245.7
14	60	20	20	150.6	98.95	282.5
15	41.6428	39.7992	18.5581	197.7	93.98	260.1
16	55	20	25	198.5	97.15	299.5
Opt	50.000	32.763	17.237	175.387	95.498	263.579

Entrapment efficiency

Entrapment efficiency was assessed by centrifugation of the nanoemulsion, followed by the collection of supernatant. The supernatant which contains the free drug was analyzed by UV spectrophotometer at 230nm.[15]

Preparation of apremilast loaded nanoemulgel

The carbopol dispersion was made initially by preparing 1% carbopol dispersion kept under magnetic stirring for 24h. Then the optimized nanoemulsion was incorporated into the carbopol dispersion and neutralized by adding triethanolamine drop until the gel was formed.

Characterization of the apremilast loaded nanoemulgel

The prepared nanoemulgel was visually examined for clarity, colour, and uniformity. Spreadability was determined by placing 0.5 g of gel on a glass plate inside a circle of 1 cm diameter pre-marked, then a second glass plate was placed over it. To the upper glass plate, a weight of 500 g was added, and the change in diameter was noted. For measuring extrudability, a collapsible tube having 20g of the gel was pressed by adding a steady weight of 1kg at the crimped end. Once the cap was removed, the amount of gel extruded from the tube was determined. Brookfield viscometer with Spindle 61 was used to determine the viscosity of nanoemulgel at 50 rpm. The pH of gel formulations was examined by using a calibrated digital pH meter.[16]

In vitro release studies

The drug release studies were carried out by positioning the cellophane membrane between the donor and receiver compartments of the Franz diffusion cell, having an area of 1.77 cm^2. The receiver compartment was filled with PBS: Methanol mixture (8:2), which acted as the release medium. The formulation was added through the donor compartment, and the whole setup was maintained at 37°C at 50 rpm. The samples were obtained from the receiver compartments at defined time intervals and replenished with an equal volume of buffer. The samples were then analyzed using a UV spectrophotometer at 230 nm to assess the drug release.[17]

Ex vivo permeation studies and flux profiles

Studies to determine *ex vivo* skin permeability were conducted using Franz diffusion cell with a diffusion area of 1.77$cm^{2,}$ and a receptor volume of 12ml under continuous magnetic stirring at 500 rpm maintained at 37±0.5°C. The receiver compartments were filled with Methanol: PBS solutions (20:80v/v, pH 6.8). Formulations were mounted on the skin with the diffusion cell covered at the top. At suitable time intervals, 2 ml aliquots were withdrawn from the receptor medium and replaced quickly by an equal volume of PBS: methanol mixture (8:2) to establish the sink conditions. The samples were analysed using a UV spectrophotometer at 230 nm. From the slope obtained from the linear portion of the cumulative drug permeated (μg/cm^2) versus time (h) curve, the flux (J) profiles were obtained. The permeability coefficient (Kp) was calculated by dividing J with the initial drug concentration in the donor cell (CO), i.e., Kp = J/CO.[18]

Skin Deposition Studies

For determining the amount of drug that remained on the skin, the Franz diffusion cell was dismantled after the permeability studies, and the skin was cautiously removed and washed multiple times with the diffusion medium to remove the excess formulation. The washings were then analyzed to estimate the amount of apremilast that remained over the skin tissue (not penetrated). To assess drug deposition, the skin tissue was sliced into tiny pieces and added to methanol for homogenization and filtered. The filtrates were centrifuged at 10,000 rpm for 15 min at 4°C. After proper dilution, the supernatant collected was analyzed for apremilast content.[19]

Accelerated stability studies

To check any physical or chemical alterations in the formulation upon storage, accelerated stability studies were conducted. The nanoemulsion was stored in amber-coloured glass vials and the nanoemulgel in collapsible aluminium tubes with caps. The formulations were subjected to three different temperatures, i.e., 8±2°C, 25±2°C, and 45±2°C/75% RH for 90 days. After 1, 2 and 3 months, the formulations were examined for changes in physical stability, droplet size, drug content, and pH.[20]

RESULTS

Solubility studies

Among the various oils, apremilast showed the highest solubility in sefsol 218 (212±0.35mg/ml); hence it was selected as the oil phase. Similarly, among surfactants, tween 80 (62.18±0.23 mg/ml) has the highest solubility and, in the case of co-surfactants, transcutol P (210±0.24 mg/ml) showed maximum solubilization. Both tween 80 and transcutol P was miscible with the oil phase. Hence tween 80 and transcutol P was selected as surfactants and co-surfactants, respectively.

Phase studies

Pseudo-ternary phase diagrams were constructed to identify the nanoemulsion area obtained after adding oil, S_{mix}, and aqueous phase. The clear and stable nanoemulsions (NE) were identified as dots in the phase diagrams, as shown in Figure 1. As observed from the phase diagrams, a large nanoemulsion gel area was obtained when tween 80 was used alone, whereas a small o/w nanoemulsion area was seen at the S_{mix} rich apex. On addition of transcutol P at a 1:1 weight ratio to tween 80, a drastic shift in the phase diagram's size and a higher nanoemulsion

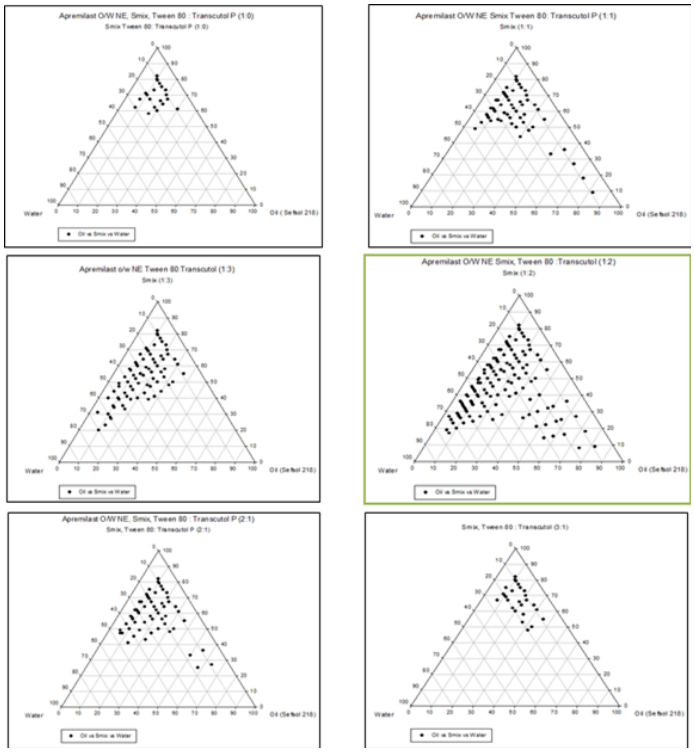

Figure 1: Pseudoternary phase diagram at different Smix ratios.

area was seen. At S_{mix} ratio of 2:1, the nanoemulsion area was less than that of the S_{mix} 1:1 ratio indicating insufficient oil phase solubilization by the S_{mix}. When analysing the S_{mix} ratio of 3:1, the phase diagram area shrank when contrasted with 2:1. As the concentration of co-surfactant was raised to the S_{mix} 1:2 level, a further increase in the nanoemulsion area by shifting the area to a water-rich region was seen compared to the S_{mix} 1:1. As the co-surfactant concentration increased further in the S_{mix} ratio, a significant reduction in the nanoemulsion area was observed.

Thermodynamic stability studies

Nanoemulsions are supposed to be stable under a wide range of temperatures and should not lose their spontaneous emulsification ability; hence thermodynamic stability studies were conducted. Some particular formulations were taken from the nanoemulsion region of the S_{mix} 1:2 phase diagram since it has the largest nanoemulsion region and were subjected to thermodynamic stability studies. None of the selected nanoemulsions demonstrated any signs of emulsion instability during thermodynamic stability tests.[21]

Optimization by D-optimal mixture design

The D-optimal mixture design is chosen for optimizing formulations containing mixtures when there are more constraints and restrictions with respect to experimental designs because it needs fewer trials as compared to other optimization designs.[22] The pre-optimization studies concluded the ranges of oil, S_{mix}, and water were 15–25%, 25–60%, and 20–60%respectively. Any variation in concentration of any of these components causes a change in the droplet size, % transmittance and solubility as described below.

Response 1 (Y1): Effect of formulation variables on particle size

It was observed that obtained droplet size was in the range of 141.7 nm to 258.1 nm. The effect of formulation variables on the droplet size can be understood from the below quadratic polynomial equation.

Particle Size = 125.823 * A + 244.421 * B + 1048.47 * C + 34.5594 * AB -818.701 * AC -1017.26 * BC

From the equation, it is understood that the linear term oil had the most significant effect on the droplet size (p<0.0001), followed by water and S_{mix}. The interaction effects of Oil and S_{mix}, as well as S_{mix} and water, decreased the globule size.

Response 2(Y2): Effect of formulation variables on percentage transmittance

The percentage transmittance of the nanoemulsions ranged from 89.05-99.85% (Table 1), indicating that all batches quickly converted to clear emulsion upon dilution. The effect of the selected variables on the percentage transmittance of the nanoemulsion can best be explained by the quadratic model equation as follows

Transmittance = 101.27* A + 88.62* B + 98.96* C- 4.75 *AB - 19.34 *AC + 0.7646 * BC

From the equation, it is clear that S_{mix} had the most significant effect on percentage transmittance (p<0.0001), followed by Oil concentration and aqueous phase concentration.

Response 3 (Y3): Effect of formulation variables solubility

The solubility is an essential factor determining drug distribution between the vehicle of nanoemulsion and stratum corneum layers. The effect of formulation factors on the solubility can be explained using the following equation.

Solubility = 274.26* A + 247.03* B + 572.42* C – 16.77* AB – 262.91* AC - 343.77* AC

The oil content (p<0.0001) in nanoemulsion formulations has been observed to have the highest positive effect on apremilast solubility, followed by S_{mix} and water content. This is due to the increased amount of solvent required to solubilize the Apremilast in the formulation.

Selection of the Optimized nanoemulsion

The optimised nanoemulsion was selected based on the criteria of achieving the lowest particle size, highest drug content, and percentage transmittance. The Optimized nanoemulsion revealed particle size of 180.60± 0.96 nm, transmittance 96.35± 0.12%, and drug content 265.2±0.63 mg/ml (Table 1) with a desirability value of 0.95, which is very close to the predicted value of particle size (175.38nm), transmittance (95.49%) and solubility (263.62mg/ml) generated by design expert software.

Characterization of optimized nanoemulsion

Particle size and PDI

The average droplet size of NE is vital as it defines the drug's release from the NE droplets. The optimized formulation was found to have a droplet size of 180.60 ± 0.96 nm. PDI of the optimized nanoemulsion is close to zero, i.e., 0.305, which implies excellent homogeneity and consistency of the formulation's droplet size.[23,24]

Zeta Potential measurement

The optimized nanoemulsion had a relatively low zeta potential of -10.08 mV, which can be attributed to the high amount of tween 80 used in formulations. The large polyoxyethylene head groups of adsorbed tween molecule, the oil droplets in the formulation are stabilized through steric repulsion against aggregation, which explains the stability improvement despite the low zeta potential.[25]

Transmission electron microscopy (TEM)

The TEM images confirmed that droplets were spherical and in the nano range (Figure 2). Due to its spherical nature, the droplets will be able to push through the minute pores of the skin and provide the required permeability.

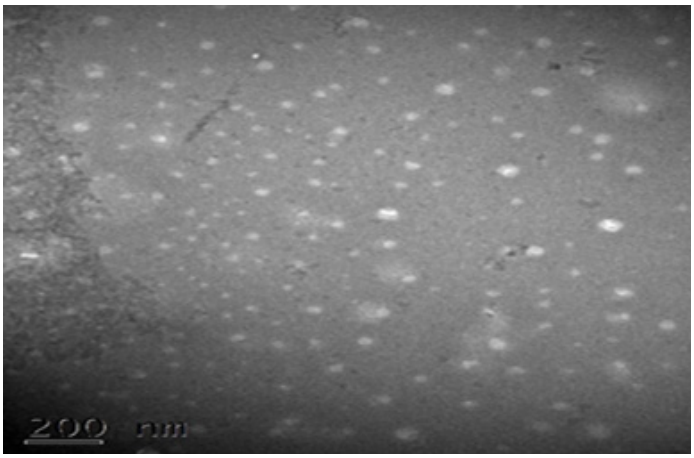

Figure 2: TEM of the optimized nanoemulsion.

Percentage transmittance

It was found that the optimized nanoemulsion has a percentage transmittance value of 96.35 ±0.28% which indicates effective emulsification of oil by S_{mix}.

Entrapment efficiency

The entrapment efficiency of the optimized nanoformulation was found to be 86.53 ±1.84%. Here, the excellent solubility of the drug in sefsol 218 and its compatibility with other components are responsible for the formulation's high entrapment efficiency. Since apremilast is insoluble in water, the drug remains entrapped inside the oil globules, which is further stabilized by surfactant and cosurfactant.[26]

Characterization of the nanoemulsion gel

Prepared nanoemulgel was clear and homogenous, with a pH of 6.6±0.23, which is acceptable for topical formulations. The formulated nanoemulgel exhibited excellent spreading properties. Hence the gels can be comfortably applied on to diseased skin since it will spread quickly. The extrudability of optimized nanoemulsion gel was observed to be 18.17±0.54, indicating the nanoemulsion gel formulation's ability to extrude out of the tube. Further, the gels were found to have an adequate viscosity of 4840±131.23cps.[27]

In vitro drug release studies

The *in vitro* release of the nanoemulsion and nanoemulgel was compared to free drug dispersion (Figure 3). The cumulative drug released from the optimized nanoemulsion (99.91%) was found to be significantly higher than drug dispersion in 24h, which is due to the nanosize of the droplets which provide a large surface area for the release of the drug, thus enhance the dissolution of apremilast into the aqueous phase and promotes the drug release.[28] The study of kinetic data showed that the release pattern meets zero-order kinetics with an R^2 value of 0.980 which implies that apremilast is released gradually at a constant rate, irrespective of the nanoemulsion's initial drug concentration. A steady quantity of the drug released over time will mitigate adverse side effects because of the decreased dosing frequency.[29]

Ex vivo permeation studies

The optimized nanoemulsion exhibited permeation of 5627±21.6µg/cm^2, whereas the nanoemulgel showed 4621±11.76 µg/cm^2 of drug permeation in 24h (Figure 4). The cumulative amount of drug permeated in 24 h through pork skin was increased by 5.56-fold in NE and 4.60 fold NEG compared to apremilast dispersion, which suggested an increase in both solubility and permeability of apremilast in NE and NEG. The flux of NE and NEG (72.5µg/cm^2/h and 42.92 µg/cm^2/h) were significantly higher than 7.98 µg/cm^2/h the drug dispersion. The permeability coefficient for NE (0.0024±0.01) and NEG (0.0014± 0.01cm^2/h) was higher as compared to dispersion (0.000232± 0.01cm^2/h). The enhancement ratio for NE and NEG was 9.12 and 5.36, respectively. NE showed a better permeation profile than the nanoemulsion gel due to the hydrogel's three-dimensional network, which delays the drug release. From the flux values, it is evident that the nanodroplets of the prepared nanoemulsions enhanced the permeation of apremilast extensively and helped cross the numerous barriers of the stratum corneum.

Ex vivo Deposition studies

The amount of apremilast deposited in the skin tissue, remained over the skin tissue and permeated through the skin is shown in Figure 5. Apremilast loaded nanoemulsion and nanoemulgel showed a percentage deposition of 66.35±1.71% and 78.85±2.38%, respectively. In the case of apremilast dispersion, it was found that a significant amount of apremilast (85.26±6.5%) was leftover the skin tissue, which is due to the low solubility and permeability of apremilast dispersion. For optimal psoriasis treatment, formulations that achieve higher drug deposition in the epidermal layer and minimum permeation through the skin are necessary for a prolonged drug release after application.[30]

Accelerated Stability Studies

The formulations showed no indications of instability like creaming, sedimentation and turbidity. Moreover, there was no much variation in the values of particle size, pH and drug content after the stability test, implying that both the formulations are stable.

DISCUSSION

The selection of the oil phase is a very critical part in formulation of nanoemulsion. The drug should be freely soluble in the oil phase as compared to surfactants. Forced solubility induced by the addition of surfactant will result in precipitation of the drug over a period of time. Sefsol 218 was able to solubilise the maximum amount of apremilast, which can be attributed to the monoglyceride chains of propylene glycol and caprylic acid, which makes it an excellent solubilizer.[31] For the rapid formation of oil in water nanoemulsions, surfactants should have high hydrophilicity and HLB value. Tween 80 with an HLB value of 15 fulfils this criterion. However, using a single surfactant, optimum transient

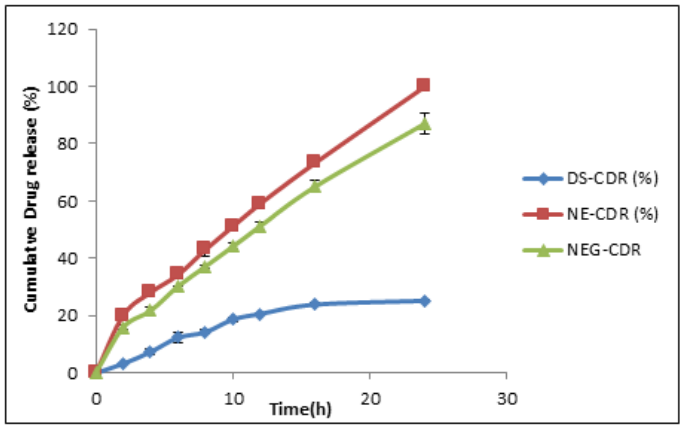

Figure 3: *In vitro* drug release studies of the apremilast loaded nanoformulations. The data represents mean ± SD with $n = 3$.

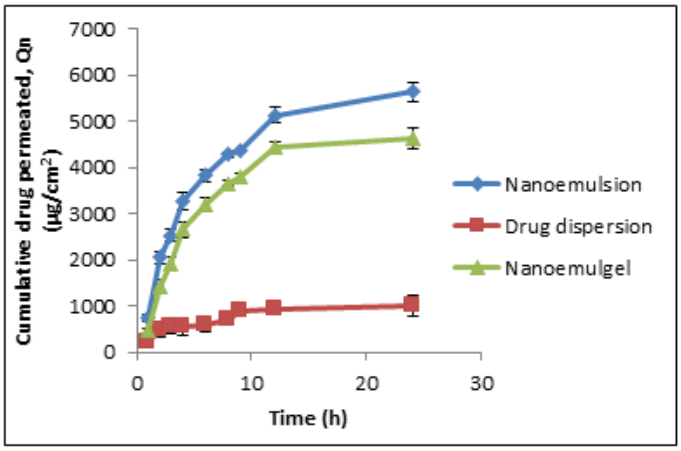

Figure 4: *Ex vivo* Permeability Studies of the apremilast loaded nanoformulations through porcine skin. The data represents mean ± SD with $n = 3$.

negative interfacial tension and fluid interfacial film cannot be obtained; hence co-surfactant is required. The inclusion of co-surfactant reduces the interface's bending stress and provides the interfacial film adequate flexibility to take up various curvatures needed to form a nanoemulsion. Thus, Transcutol-P with the HLB value of 4.2 was chosen as the co-surfactant.[32]

From the phase diagrams, it was observed that the nanoemulsion area in the absence of transcutol was minimal; this is because the oil phase has been solubilized to a lesser extent. This suggests that the surfactant alone was not adequate to decrease the oil droplet's interfacial tension to a sufficiently low level and is unable to decrease the system's free energy to the ultra-low level required to form nanoemulsions. Transcutol P, when added at a 1:1 ratio, further reduced the interfacial tension and increased fluidity of the interface, thus increased the nanoemulsion region. At 2:1, shrinkage in the nanoemulsion area was seen due to insufficient oil phase solubilization by the S_{mix}. On further increasing the tween 80 concentration, the nanoemulsion region reduced, thereby indicating that the least interfacial tension and optimum curvature had been reached. However, when the co-surfactant ratio was increased to 1:2, the maximum nanoemulsion region was seen, which can be attributed to transcutol P's superiority in nanosizing. On further increasing the co-surfactant ratio, substantial shrinkage was observed in the nanoemulsion area. It is attributable to the liquid crystalline phase produced by Transcutol P, which was not stabilized by the given tween 80 amount.[31,33]

The three ratios, 1:1, 2:1, 1:2 produced stable and transparent nanoemulsions from each phase diagram constructed; however, it was found that the S_{mix} ratio of 1:2 showed the highest area of nanoemulsion. Hence this ratio was taken for further formulation development.

The concentration of oil, S_{mix} and water was optimised by employing Box-Behnken design with the help of design expert software. The emulsion's droplet size is a crucial factor since it defines the rate and extent of drug permeation. As observed from the counterplot (Figure 6), the smallest droplet size was obtained at lower oil levels by increasing S_{mix}. Raising the S_{mix} content at lower levels of oil allowed a better reduction in oil/water interfacial tension resulting in a decrease in globule size, thus least globule sizes were achieved at the lowest levels of oil and moderate to high levels of surfactant. It was found that higher levels of water and oil content induced a rise in globular size values as growing water content eliminates the rigid film, which contributes to droplet coalescence and increased droplet size. Whereas at higher oil levels, the S_{mix} does not emulsify the oil as effectively as it did when less oil was used[34,35]

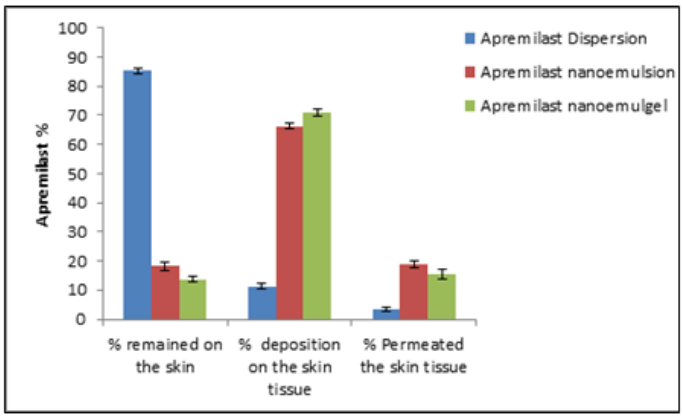

Figure 5: *Ex vivo* Deposition studies of the apremilast loaded nanoformulations carried out in Porcine skin. The data represents mean ± SD with $n = 3$.

The high percentage transmission for the formulation demonstrates the oil phase's efficient emulsification into the aqueous phase. In the contour plot (Figure 6), it was observed at moderate to higher surfactant levels that percentage transmittance of the nanoemulsion was maximum, especially at low oil content levels. This is because, at low levels of oil and high surfactant levels, efficient emulsification of oils into small globules occurs, resulting in high transmittance. It was observed that at high water content levels, transmittance was reduced, especially when oil levels are high, a phenomenon which was prevalent along with decreasing surfactant levels, indicating inefficient emulsification of oil into nanosized globules.[36] The contour plot of solubility (Figure 6) shows that Both oil and S_{mix} had a significant effect on the drug's solubility. The incorporation of S_{mix} into nanoemulsions results in high solubilization capacity allowing a large number of the drug to be incorporated. From the results, it can be summarised that sefsol oil and S_{mix} are mainly responsible for the formulation's enhanced drug content.[37]

The optimised formulation had particle size in the nano range and low polydispersity index, due to which the formulation can easily permeate the skin. The nanoemulsion size appeared as a dark spherical shaped bubble on bright surroundings in the transmission electron microscope, and the droplet size was comparable to the size obtained using the Malvern zeta sizer. The pH of nanoemulsion gel was within the skin pH range and will not cause any skin reactions.

In the process of biological permeation, the drug should first release from the vehicles, and then it can be partitioned into or absorbed by the skin. Thus, *in vitro* drug release studies were conducted to ensure whether the drug is adequately released from formulations to meet the requirement. The prolonged drug release of the drug for 24hr seen in case of nanoemulsions and nanoemulgel may be attributed to the presence of tween 80, which exhibits higher emulsifying ability resulting in nano-

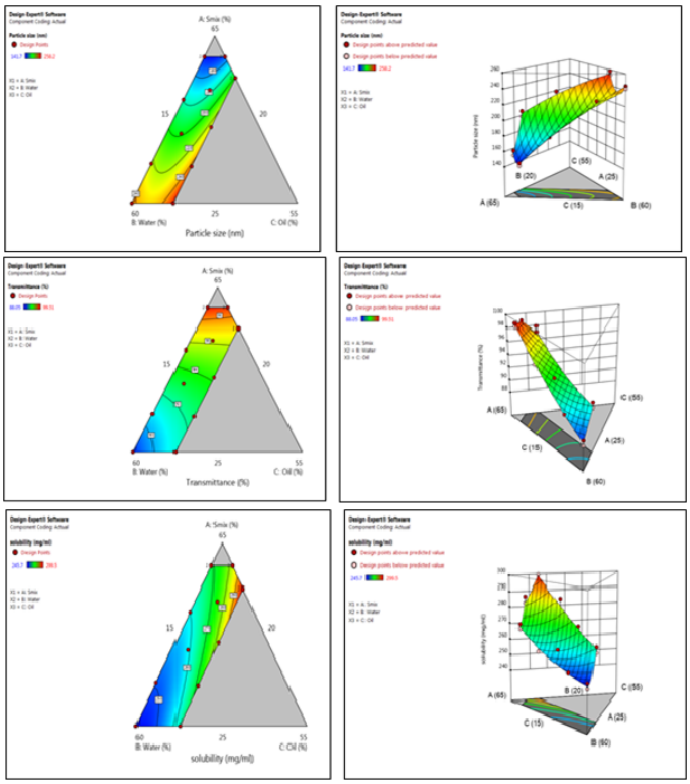

Figure 6: The 2D surface and 3D surface plots illustrated effect of three variables: Smix (A), water (B) and oil (C) on the Particle size, Percentage transmittance and Solubility of nanoemulsion.

level droplet size, which provides a large surface area for the release of the drug, thus promotes the rate of dissolution of apremilast and permits the drug release. Additionally, in the case of nanoemulgel, drug release of the drug is further sustained due to the three dimensional hydrogel matrix and high viscosity of the gel.[38]

Both skin permeation and deposition studies have shown that a significant amount of apremilast was able to permeate and get deposited in the skin compared to drug dispersion. The increased flux and permeation coefficient obtained from nanoemulsion and nanoemulgel is due to the nano size of the droplets, which increases the solubilization of the drug and enables it to permeate the different layers of the skin easily. Further, the liquid lipid employed in the formulation helps in the fluidization of the phospholipid bilayer in the stratum corneum.[39]

Psoriatic skin is crucial to treat due to the hyper-proliferation of the epidermal keratinocytes and requires topical formulation rather than transdermal. As per the requirement of an ideal formulation, higher deposition of drug in epidermal skin and minimal permeation through the skin is required to release the drug for a more extended period after application. The developed apremilast nanoemulsion fulfils this requirement and can be considered for topical psoriasis therapy after conducting further preclinical and clinical studies.[40]

CONCLUSION

The optimum outcome in psoriasis therapy can be achieved by formulations that achieve maximum skin deposition with minimal permeation. The developed nanoemulgel fulfilled the above criteria besides being thermodynamically stable. However, further *in vivo* study need to be carried out to confirm the anti-psoriatic activity of the developed nanoemulgel.

ACKNOWLEDGEMENT

The authors are thankful to NGSM Institute of Pharmaceutical sciences, Mangalore, for providing the facilities to carry out the research work. The authors are also grateful to Glenmark Life sciences, Ankleshwar, Gujarat, for providing the gift sample.

ABBREVIATIONS

PDI: Polydispersibility index; **TEM:** Transmission electron microscopy; **NE:** Nanoemulsion; **NEG:** Nanoemulgel; **PBS:** Phosphate buffer saline; **S_{mix}:** Ratio of oil surfactant and co-surfactant.

REFERENCES

1. McCormick T, Ayala-Fontanez N, Soler D. Current knowledge on psoriasis and autoimmune diseases. Psoriasis Targets Ther. 2016;6:7-32.
2. Chong HT, Kopecki Z, Cowin AJ. Lifting the silver flakes: The pathogenesis and management of chronic plaque psoriasis. Biomed Res Int. 2013;168321:1-9.
3. Thappa D, Malathi M. Topical therapy of psoriasis: Where do we stand?. J Postgrad Med. 2017;63(4):210-2.
4. Pradhan M, Singh D, Singh MR. Novel colloidal carriers for psoriasis: Current issues, mechanistic insight and novel delivery approaches. J Control Release. 2013;170(3):380-95.
5. Bubna AK. Apremilast: A dermatologic perspective. Indian J Drugs Dermatol. 2016;2(2):72-82.
6. Kushwaha AS, Repka MA, Murthy NS. A Novel Apremilast Nail Lacquer Formulation for the Treatment of Nail Psoriasis. AAPS Pharm Sci Tech. 2017;18(8):2949-56.
7. Pund S, Pawar S, Gangurde S, Divate D. Transcutaneous delivery of leflunomide nanoemulgel: Mechanistic investigation into physicomechanical characteristics, *in vitro* anti-psoriatic and anti-melanoma activity. Int J Pharm. 2015;487(1-2):148-56.
8. Musa SH, Basri M, Masoumi HRF, Shamsudin N, Salim N. Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: *In vitro* diffusion and *in vivo* hydrating action. Int J Nanomedicine. 2017;12:2427-41.
9. Kaur A, Katiyar SS, Kushwah V, Jain S. Nanoemulsion loaded gel for topical co-delivery of clobitasol propionate and calcipotriol in psoriasis. Nanomedicine Nanotechnology, Biol Med. 2017;13(4):1473-82.
10. Gupta AK, Mishra DK, Mahajan SC. Preparation and *in-vitro* evaluation of self emulsifying drug delivery system of antihypertensive drug valsartan. Int J Pharm Life Sci. 2011;2(3):633-9.
11. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. Int J Pharm Investig. 2012;2(3):140-9.
12. Kaur R, Ajitha M. Formulation of transdermal nanoemulsion gel drug delivery system of lovastatin and its *in vivo* characterization in glucocorticoid induced osteoporosis rat model. J Drug Deliv Sci Technol. 2019;52:968-78.
13. Kaur R, Ajitha M. Transdermal delivery of fluvastatin loaded nanoemulsion gel: Preparation, characterization and *in vivo* anti-osteoporosis activity. Eur J Pharm Sci. 2019;136(104956):1-10.
14. Aqil M, Kamran M, Ahad A, Imam SS. Development of clove oil based nanoemulsion of olmesartan for transdermal delivery: Box-Behnken design optimization and pharmacokinetic evaluation. J Mol Liq. 2016;214:238-48.
15. Shofia SI, Jayakumar K, Mukherjee A, Chandrasekaran N. Efficiency of brown seaweed (*Sargassum longifolium*) polysaccharides encapsulated in nanoemulsion and nanostructured lipid carrier against colon cancer cell lines HCT 116. RSC Adv. 2018;8(29):15973-84.
16. Gaba B, Fazil M, Khan S, Ali A, Baboota S, Ali J. Nanostructured lipid carrier system for topical delivery of terbinafine hydrochloride. Bull Fac Pharmacy, Cairo Univ. 2015;53(2):147-59.
17. Kajbafvala A, Salabat A, Salimi A. Formulation, characterization, and *in vitro/ex vivo* evaluation of quercetin-loaded microemulsion for topical application. Pharm Dev Technol. 2018;23(8):741-50.
18. Divya G, Panonnummal R, Gupta S, Jayakumar R, Sabitha M. Acitretin and aloe-emodin loaded chitin nanogel for the treatment of psoriasis. Eur J Pharm Biopharm. 2016;107:97-109.
19. Pandey SS, Maulvi FA, Patel PS, Shukla MR, Shah KM, Gupta AR, *et al.* Cyclosporine laden tailored microemulsion-gel depot for effective treatment of psoriasis: *In vitro* and *in vivo* studies. Colloids Surf B Biointerfaces. 2020;186(110681):1-28.
20. Ali MS, Alam MS, Alam N, Anwer T, Safhi MMA. Accelerated stability testing of a clobetasol propionate-loaded nanoemulsion as per ICH guidelines. Sci Pharm. 2013;81(4):1089-100.
21. Chouhan P, Saini TR. D-optimal design and development of microemulsion based transungual drug delivery formulation of ciclopirox olamine for treatment of onychomycosis. Indian J Pharm Sci. 2016;78(4):498-511.
22. Rao S, Barot T, Rajesh KS, Jha LL. Formulation, optimization and evaluation of microemulsion based gel of Butenafine Hydrochloride for topical delivery by using simplex lattice mixture design. J Pharm Investig. 2016;46(1):1-2.
23. Sutradhar KB, Amin L. Nanoemulsions: Increasing possibilities in drug delivery. Eur J Nanomed. 2013;5(2):97-110.
24. Sari TP, Mann B, Kumar R, Singh RRB, Sharma R, Bhardwaj M, *et al.* Preparation and characterization of nanoemulsion encapsulating curcumin. Food Hydrocoll. 2015;43:540-6.
25. Hashem FM, Shaker DS, Ghorab MK, Nasr M, Ismail A. Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. AAPS Pharm Sci Tech. 2011;12(3):879-86.
26. Panonnummal R, Jayakumar R, Sabitha M. Comparative anti-psoriatic efficacy studies of clobetasol loaded chitin nanogel and marketed cream. Eur J Pharm Sci. 2017;96:193-206.
27. Rajitha P, Shammika P, Aiswarya S, Gopikrishnan A, Jayakumar R, Sabitha M. Chaulmoogra oil based methotrexate loaded topical nanoemulsion for the treatment of psoriasis. J Drug Deliv Sci Technol. 2019;49:463-76.
28. Miastkowska M, Sikora E, Ogonowski J, Zielina M, Łudzik A. The kinetic study of isotretinoin release from nanoemulsion. Colloids Surfaces A Physicochem Eng Asp. 2016;510:63-8.
29. Madan JR, Khobaragade S, Dua K, Awasthi R. Formulation, optimization, and *in vitro* evaluation of nanostructured lipid carriers for topical delivery of Apremilast. Dermatol Ther. 2020;33(3):1-32.
30. Sharma S, Sahni JK, Ali J, Baboota S. Effect of high pressure homogenization on formulation of TPGS loaded nanoemulsion of rutin—pharmacodynamic and antioxidant studies. Drug Deliv. 2015;22(4):541-51.
31. Ahmad J, Gautam A, Komath S, Bano M, Garg A, Jain K. Topical Nano-emulgel for Skin Disorders: Formulation Approach and Characterization. Recent Pat Antiinfect Drug Discov. 2018;14(1):36-48.
32. Censi R, Martena V, Hoti E, Malaj L, Martino DP. Permeation and skin retention of quercetin from microemulsions containing Transcutol®P. Drug Dev Ind Pharm. 2012;38(9):1128-33.
33. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm. 2007;66(2):227-43.
34. Kumar N, Shishu. D-optimal experimental approach for designing topical microemulsion of itraconazole: Characterization and evaluation of antifungal efficacy against a standardized Tinea pedis infection model in Wistar rats. Eur J Pharm Sci. 2015;67:97-112.

35. Duangjit S, Mehr LM, Kumpugdee-Vollrath M, Ngawhirunpat T. Role of simplex lattice statistical design in the formulation and optimization of microemulsions for transdermal delivery. Biol Pharm Bull. 2014;14(59):1-32.
36. Barot BS, Parejiya PB, Patel HK, Gohel MC, Shelat PK. Microemulsion-based gel of terbinafine for the treatment of onychomycosis: Optimization of formulation using D-optimal design. AAPS Pharm Sci Tech. 2012;13(1):184-92.
37. Golmohammadzadeh S, Farhadian N, Biriaee A, Dehghani F, Khameneh B. Preparation, characterization and *in vitro* evaluation of microemulsion of raloxifene hydrochloride. Drug Dev Ind Pharm. 2017;43(10):1619-25.
38. Elmataeeshy ME, Sokar MS, Bahey-El-Din M, Shaker DS. Enhanced transdermal permeability of Terbinafine through novel nanoemulgel formulation; Development, *in vitro* and *in vivo* characterization. Futur J Pharm Sci. 2018;22(6):591-9.
39. Sharma B, Iqbal B, Kumar S, Ali J, Baboota S. Resveratrol-loaded nanoemulsion gel system to ameliorate UV-induced oxidative skin damage: from *in vitro* to *in vivo* investigation of antioxidant activity enhancement. Arch Dermatol. 2019;311(10):773-93.
40. Kaur N, Sharma K, Bedi N. Topical Nanostructured Lipid Carrier Based Hydrogel of Mometasone Furoate for the Treatment of Psoriasis. Pharm Nanotechnol. 2018;6(2):133-43.

Article History: Submission Date : 29-01-2021; Revised Date : 23-03-2021; Acceptance Date : 11-05-2021.
Cite this article: Mulleria SS, Koland M, Ghetia SM. Formulation, Optimization and *in vitro* Evaluation of Apremilast Nanoemulgel for Topical Delivery. Int. J. Pharm. Investigation, 2021;11(2):230-7.